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Brief Articles

Targeted Pathologic Evaluation of Bone Marrow Donors Identifies Previously Undiagnosed Marrow Abnormalities

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A B S T R A C T

Potential bone marrow donors are screened to ensure the safety of both the donor and recipient. At our institution, potential donors with abnormal peripheral blood cell counts, a personal history of malignancy, or age >60 years are evaluated to ensure that they are viable candidates for donation. Evaluation of the marrow includes morphologic, flow cytometric, and cytogenetic studies. A total of 122 potential donors were screened between the years of 2001and 2011, encompassing approximately 10% of all donors. Of the screened potential donors, the mean age was 59 years and there were 59 men and 63 women. The donors were screened because of age >60 years (n = 33), anemia (n = 22), cytopenias other than anemia (n = 27), elevated peripheral blood counts without a concurrent cytopenia (n = 20), elevated peripheral blood counts with a concurrent cytopenia (n = 10), history of malignancy (n = 4), abnormal peripheral blood differential (n = 3), prior graft failure (n = 1), history of treatment with chemotherapy (n = 1), and body habitus (n = 1). Marrow abnormalities were detected in 9% (11 of 122) of donors. These donors were screened because of anemia (5 of 22, 23%), age >60 years (2 of 33, 6%), history of malignancy (2 of 4, 50%), elevated peripheral blood counts (1 of 20, 5%), and body habitus (1 of 1, 100%). Abnormalities included plasma cell dyscrasia (n = 3), abnormal marrow cellularity (n = 3), clonal cytogenetic abnormalities (n = 2), low-grade myelodysplastic syndrome (1), a mutated JAK2 V617F allele (n = 1), and monoclonal B cell lymphocytosis (n = 1). Our experience indicates that extended screening of potential donors identifies a significant number of donors with previously undiagnosed marrow abnormalities.

INTRODUCTION

Allogeneic blood or marrow transplantation (BMT) is the definitive therapy for a variety of lymphoproliferative disorders, leukemias, and other conditions affecting the bone marrow, such as sickle cell anemia. Transplantation-related mortality continues to decrease because of improvements in immunosuppressive regimens and conditioning protocols, as well as advances in HLA typing, and BMT is now a viable therapy option for increasing numbers of patients. As a result of these advances, the ages of both BMT recipients and donors have increased [1].

The use of older bone marrow donors carries increased risk for both the donor and recipient. The risks for older donors include an increased likelihood of medical complications during the allograft collection and prolonged lymphopenias after hematopoietic stem cell donation [2]. Older donors are also more likely to have underlying medical conditions, which increase the risk of transmitting a disorder from the donor to the recipient.

The transmission of disease from a BMT donor to recipient is rare but well documented, and diseases that may be transmitted by BMT include genetic disorders, infections, autoimmune diseases, and both hematologic and non-hematologic malignancies [3-11]. Although once thought to be a rare occurrence, "donor cell leukemia" is being reported with increased frequency, with recent estimates suggesting it may account for up to 5% of all leukemia "relapses" [12-18]. In addition, there are reports of lymphoma and myelodysplastic syndrome (MDS) that were transferred from donor to recipient as a result of BMT [19-21]. The reasons for the apparent recent increase in donor-derived malignancies after allogeneic BMT are probably multifactorial and include better recognition through modern molecular techniques, as well as the use of older donors.

Although many medical disorders in donors are easily identified through routine laboratory testing, some hematologic disorders can be more challenging to identify, especially when peripheral blood counts remain within normal limits. This is particularly true of low-grade or indolent disorders, including plasma cell dyscrasia/monoclonal gammopathy of undetermined significance, monoclonal B lymphocytosis (MBL), and low grade MDS. Although the risks of these conditions developing into clinically significant disease after transplantation are not known, in most reported cases of donor-recipient transmission of hematologic malignancies, the donor abnormality was discovered when the disease progressed and was diagnosed in the recipient.

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Table 1
Donor Screening Results

Characteristic	Donors	Eliminated Donors	Diagnosis
Totals	122	11 (9%)	
Age, median (range), yr	59 (17-80)	62 (40-77)	
Males	59	6 (10% of males)	
Females	63	5 (8% of females)	
Reason for evaluation			
>60 yr old	33	2 (6%)	Hypercellular marrow (n = 1) Abnormal karyotype (n = 1)
Anemia	22	5 (23%)	Plasma cell dyscrasia (n = 2) Low-grade myelodysplastic syndrome (n = 1) Hypocellular marrow (n = 1) Hypercellular marrow (n = 1)
Elevated counts without concurrent cytopenia	20	1 (5%)	Mutated JAK2 V617F
History of malignancy	4	2 (50%)	Monoclonal B lymphocytosis (n = 1) Abnormal karyotype (n = 1)
Body habitus	1	1 (100%)	Plasma cell dyscrasia
Cytopenia(s) other than anemia	27	0	
Elevated counts with concurrent cytopenia	10	0	
Abnormal peripheral blood differential	3	0	
Prior graft failure	1	0	
History of treatment with chemotherapy	1	0	

At our institution, all potential bone marrow donors are screened with a comprehensive work-up to ensure the safety of both the donor and the recipient. The screening includes peripheral blood counts, physical examination, and a chest x-ray. Potential donors with abnormal peripheral blood cell counts, a personal history of malignancy, and those over 60 years of age undergo more comprehensive testing for hematologic disorders, including a bone marrow biopsy with morphologic, flow cytometric, molecular, and cytogenetic analyses. From 2001 to 2011, 122 potential donors, representing approximately 10% of all donors, were evaluated with a bone marrow aspirate and biopsy at our institution. This study reviews the results of the extended screening of these bone marrow donors. We find that pathologic evaluation of bone marrow allows the identification of a significant population of donors with previously unrecognized bone marrow abnormalities.

MATERIALS AND METHODS

Donor Identification

The pathology database was searched using the keywords *marrow* and *donor*, and 122 potential donors who were evaluated with a bone marrow aspirate and biopsy were identified. Clinical data were reviewed by one of the authors (A.S.D.) in accordance with IRB-approved protocol NA_00051476.

Histology

Hematoxylin and eosin slides from formalin-fixed and paraffin-embedded bone marrow core biopsies were reviewed along with Wright-Giemsa–stained aspirate smears. Any associated immunohistochemical stains performed at the time of the original diagnosis were also reviewed, but no additional stains were done solely for the purposes of this study.

Flow Cytometry

Flow cytometric immunophenotyping was performed on fresh bone marrow aspirates. The material was collected in EDTA or heparin anticoagulant and processed routinely using a red cell lysis method. Cell suspensions were incubated with combinations of 4 monoclonal antibodies (Becton Dickinson,) that were used at concentrations titrated for optimal staining. In most cases the panel included antibodies specific for CD45, CD71, HLA-DR, CD33, CD13, CD2, CD3, CD5, CD7, CD19, CD20, kappa light chain, lambda light chain, CD22, CD10, CD34, CD56, CD38, CD14, CD64, CD61, CD11b, CD15, CD117, and CD10, although occasional specimens were subjected to an abbreviated panel. Selected antibody combinations were conjugated to fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, and allophycocyanin fluorochromes. Specimens were analyzed on a BDIS FACSCalibur flow cytometry system (Becton Dickinson FACSCalibur, San Jose, California).

List mode data files were acquired and analyzed for each specimen using CellQuest and Paint-a-Gate software programs, respectively (Becton Dickinson). An antigen was considered positive if the cells of interest either showed a homogeneous distribution with the median intensity at least 20 log channels above that seen in the control or if there was a heterogeneous distribution of antigen expression, such that a subpopulation of cells was above that seen in the control. Cell populations were classified as dim, moderate, and bright, based on their intensity compared with normal counterpart cell populations. All flow cytometric data were reviewed by 2 of the authors (M.T. and A.S.D.).

Cytogenetics

Cells from the bone marrow biopsies were cultured without mitogens using current techniques and subsequently harvested. Slides were prepared and G-banded using standard techniques. At least 20 metaphase cells were analyzed per specimen.

Fluorescence In-Situ Hybridization (FISH) Analysis

FISH analysis included probes 5p15.2 (D5S23, D5S23), 5q31 (EGR1), 7cen (D7Z1), 7q31 (D7S522), 8cen (D8Z2), 11q23 (MLL), and 20q12 (D20S108) (Abbott Molecular, Des Plaines, Illinois). After FISH, 200 interphase nuclei were scored for loci on 5p15.2, 5q31, 7 centromere, 7q31, 8 centromere, and 11q23. For 200 nuclei, the normal cutoffs were as follows: <2.5% for monosomy 5, <3.5% for del(5q), <4.4% for monosomy 7, <4.8% for del(7q), <6.7% for trisomy 8, and <3% for rearrangement of 11q23. Five hundred interphase nuclei were scored for the locus on 20q12. For 500 nuclei, the normal cutoff for monosomy/del(20q) is <6%.

Table 2
Pathologic Findings in Abnormal Donors

Age, y/Sex	Reason for Biopsy	Marrow Morphology	Flow Cytometry	Cytogenetic/Molecular Findings	Diagnosis	Intended Recipient Outcome
62/F	>60 yr of age	Hypercellular (~60%) with increased megakaryocytes, some of which are small with hypolobated nuclei	N/D	FISH: normal Karyotype: normal		Underwent transplantation using another family member as a donor
72/F	>60 yr of age	Mild hypercellularity (~60%)	Normal	FISH: normal Karyotype: del(5q) [8/20]		Underwent transplantation using another family member as a donor
72/M	Anemia (HCT: 37.2%)	Normocellular with <5% kappa light chain-restricted plasma cells	N/D	FISH: normal Karyotype: N/D	Plasma cell dyscrasia	Patient died of disease as potential donors were being identified
77/F	Anemia (HCT: 33.9%)	Slightly hypercellular (40% to 50%) with <10% monoclonal λ-restricted plasma cells	4% λ -restricted plasma cells	FISH: normal Karyotype: normal	Plasma cell dyscrasia	No other potential donors identified; autologous transplantation performed
43/F	Anemia (HCT: 22.6%)	Hypercellular (100%) with atypical megakaryocytes & dysplastic myeloid maturation	N/D	FISH: normal Karyotype: normal	Low-grade myelodysplastic syndrome	Transplantation cancelled because of disease progression
72/M	Anemia (HCT: 35.9%)	Hypercellular (50% to 60%) with occasional small megakaryocytes with hypolobated nuclei	Normal	FISH: normal Karyotype: normal		Underwent transplantation using another family member as a donor
46/M	Anemia (HCT: 39.4%)	Hypocellular (~20%)	Normal	FISH: normal Karyotype: normal		Transplantation cancelled because of disease progression
40/M	Elevated platelets (411 K/cu mm)	Normal	Normal	Mutated JAK2 V617F allele FISH: normal Karyotype: normal		Patient refused transplantation
71/M	History of renal cell carcinoma	Hypercellular (60% to 70%) with scattered B lymphocytes	(2%) monoclonal B lymphocytes (CD20-bright, CD5-, CD10-, λ+)	FISH: normal Karyotype: balanced t(2;3), -Y	Monoclonal B lymphocytosis	Transplantation on hold because of excellent response to chemotherapy; partially matched unrelated donor was identified if needed
45/M	History of testicular cancer	Normal	Normal	FISH: del(20q) (10%) Karyotype: del(20q) [3/21]		Underwent transplantation using another family member as a donor
54/F	Body habitus	Normal	Few CD56+, κ-restricted plasma cells	FISH: normal Karyotype: normal	Plasma cell dyscrasia	Transplantation cancelled because of disease progression

N/D indicates that the test was not done; HCT, hematocrit.

RESULTS

The potential donors included 59 men and 63 women. The median age of the screened potential donors was 59 (range, 17 to 80) years. The indications for marrow evaluation in the donors are summarized in Table 1 and include the following: age > 60 years, anemia, elevated counts without a concurrent cytopenia, history of malignancy, body habitus (obesity), cytopenias other than anemia, elevated counts with a concurrent cytopenia, abnormal peripheral blood differential, prior graft failure, and history of treatment with chemotherapy. Eleven (9%) of the potential donors who were screened were rejected because of abnormalities found upon pathologic examination of the marrow. The reasons for bone marrow evaluation on these rejected donors included anemia (n = 5), history of malignancy (n = 2), age > 60 years (n = 2), elevated peripheral blood counts (n = 1), and body habitus (n = 1). Atypical findings in the 11 donors were detected by morphologic examination of the marrow (n = 5), flow cytometric studies (n = 1), both biopsy and flow cytometry (n = 2), molecular studies (n = 1), cytogenetic studies (n = 1), and both biopsy and cytogenetic studies (n = 1) (Table 2).

Thirty-three donors underwent extended screening solely because they were over 60 years old, and 2 potential donors (2 of 33, 6%) were eliminated. A 62-year-old woman had hypercellular (60%) marrow for her age with increased morphologically atypical megakaryocytes. Of note, there was no evidence of dysplasia on the aspirate and cytogenetic studies were normal. Another 72-year-old female donor also showed slightly hypercellular marrow with nonspecific changes on morphologic examination of the biopsy, but a karyotype showed a clonal population of cells with del(5q). Metaphase FISH with a probe mapped at 5q31 failed to show the deletion as the probe is located outside the deleted segment. Neither of these potential donors had cytopenias; however, they were excluded from the donor pool because of concern for an evolving primary marrow disorder.

Five of 22 (23%) potential donors with anemia were found to have bone marrow abnormalities. Two donors were diagnosed with plasma cell dyscrasia. Both donors had a relatively mild anemia; a 72-year-old man had a hematocrit of 37.2% (normal male range: 41% to 53%), and a 77-year-old woman had a hematocrit of 33.9% (normal female range: 36% to 46%). The diagnosis was identified by marrow biopsy in the male donor, and by biopsy and flow cytometric analysis in the female donor's marrow. A third potential donor was a 43-year-old woman with a hematocrit of 22.6%. Morphologic examination of the biopsy and aspirate showed a markedly hypercellular marrow with atypical megakaryocytes and dysplastic myeloid maturation, consistent with a low-grade myelodysplastic syndrome. Cytogenetic studies were normal in this potential donor. The remaining 2 donors with anemia were excluded because of marrow abnormalities, although the findings were not diagnostic of a specific primary marrow disorder. Both of these donors were men with only a mild decrease in hematocrit. A 72-year-old potential donor with a hematocrit of 35.9% had abnormally hypercellular (50% to 60%) marrow on biopsy, and a 46-year-old potential donor had abnormally hypocellular (about 20%) marrow.

Twenty potential donors had elevated peripheral blood counts with no concurrent cytopenia, and 1 (1 of 20, 5%) of these donors was found to have a marrow abnormality. This healthy 40-year-old man had normal peripheral blood counts with the exception of a slightly elevated platelet count (411 K/cu mm, normal range: 150 K/cu mm to 350 K/cu mm). Morphologic, flow cytometric, and cytogenetic studies were normal, but molecular testing demonstrated the presence of at least 1 mutated JAK2 V617F allele.

Four donors were evaluated because of a history of malignancy; the potential donors had a history of prostate cancer, breast cancer, testicular cancer, and renal cell carcinoma. Two (2 of 4, 50%) potential donors were eliminated because of marrow findings. A 45-year-old man with a history of testicular cancer who was treated with chemotherapy was found to have a clonal population of cells that showed a deletion of chromosome 20q by FISH and metaphase analysis. The second eliminated donor was a 71-year-old man with a history of renal cell carcinoma, although the details of his treatment for renal cell carcinoma are unknown. His bone marrow was hypercellular. An immunostain for CD20 showed scattered B cells, and flow cytometry studies demonstrated a small population of monoclonal lymphocytes that expressed bright CD20 and were negative for CD5 consistent with a monoclonal B lymphocytosis.

Finally, 1 potential donor, an obese 54-year-old woman, was found to have abnormalities on flow cytometric analysis of the marrow. This donor had normal blood counts and an unremarkable medical history, and a bone marrow biopsy was performed to determine whether a bone marrow harvest was anatomically feasible. Although the morphologic and cytogenetic studies were normal, flow cytometric analysis demonstrated a small population of plasma cells that expressed kappa light chain and aberrant CD56, consistent with a plasma cell dyscrasia.

None of the potential donors who underwent extended marrow evaluation due to cytopenias other than anemia (n = 27), elevated counts with a concurrent cytopenia (n = 10), abnormal peripheral blood differential (n = 3), prior graft failure (n = 1), and history of treatment with chemotherapy (n = 1) demonstrated marrow abnormalities.

Four of the potential donors with peripheral blood count abnormalities had normal findings on the marrow studies but were eliminated from consideration because of other reasons. Chest x-rays revealed adenocarcinoma in the lung in a 61-year-old man with anemia, as well as a mediastinal mass in a 38-year-old man with lymphopenia. A 63-year-old man with anemia was eliminated because he had blood in his stool, raising concern for a potential gastrointestinal malignancy. Finally, a 21-year-old man with elevated hemoglobin and a mild leukopenia was found to have HLA antibodies against the potential recipient.

DISCUSSION

Thorough and effective pretransplantation screening of potential allogeneic bone marrow donors is essential to ensure the best outcome for both the donor and the recipient. Pretransplantation donor screening procedures are not standardized, but elements of the work-up are common to most institutions, including a thorough medical history and physical examination, laboratory studies with a complete blood count and blood chemistries, testing for transmissible diseases, an electrocardiogram, and chest x-ray. Sampling and examination of a potential donor's bone marrow is often not routinely performed.

At our institution, we began performing bone marrow evaluation, including morphology, cytogenetics, and flow cytometry, on potential donors who were over 60 years old and had abnormal peripheral blood counts or a personal history of malignancy. In this study we evaluated the results of screening these donors over the past 10 years. We found that 11 of 122 (9%) of donors had hematologic abnormalities

that warranted reconsideration of the use of their marrow, and as a result of these findings we have begun routinely testing all potential donors who meet the aforementioned criteria.

Most of the donors in whom abnormalities were found either had a prior disease history or evidence of peripheral blood count abnormalities; features that most would accept, warrant more extensive investigation. However, 2 of 33 donors screened based on age alone also had marrow findings that precluded their donating. Although our institution utilizes 60 years of age as a cut-off for extended screening, other authors have suggested that donors over 55 years old should be screened [1]. These limits are relatively arbitrary, and the potential for even younger donors to have an undetected marrow abnormality is demonstrated by the plasma cell dyscrasia that was identified in a 54-year-old potential donor with normal peripheral blood counts who was evaluated because of her body habitus. Although unexpected marrow abnormalities can be found even in younger donors with an unremarkable medical history, current data are limited and, at this time, it is not possible to determine the optimal age to begin routine extended screening.

The marrow evaluations performed in this study tended to identify relatively minor abnormalities. Of the 11 donors who were rejected, only 4 were given a definitive diagnosis of a hematologic abnormality including plasma cell dyscrasia (n = 3), MBL (n = 1), and low-grade MDS (n = 1). The remaining donors were rejected because of either morphologic abnormalities in the marrow including hypercellularity (n = 2) or hypocellularity (n = 1), a JAK V617F mutation (n = 1), or clonal cytogenetic abnormalities that did not meet the criteria for a diagnosis of MDS (n = 2). Although in theory rejecting these donors can potentially benefit the recipient by reducing the possibility of a donor transmitted neoplasm, in practice, the risks of transmitting such a neoplasm are largely unknown. Plasma cell dyscrasia and MBL, in particular, rarely progress to overt malignancy, although it is not clear what effects the stress of hematologic reconstitution could have on these conditions [22]. Moreover, finding unexpected abnormalities can trigger anxiety in the potential donor who now requires monitoring for laboratory findings that may never evolve into a full-fledged neoplasm. Other potential disadvantages of marrow evaluation in the screening of potential bone marrow donors include increased cost, possible morbidity, and increased reluctance of candidates to undergo the screening process [23]. For all these reasons, extensive donor screening should be undertaken carefully, and decisions about what to do with the results must be made in context of individual patient circumstances [24].

One possible concern is that screening could eliminate the only available donor for a patient. One patient in this study did not undergo a BMT because his donor was eliminated, and the patient ultimately died. Of note, this donor was eliminated in 2001, and a similar outcome would be unlikely today. Recent advances now demonstrate that unrelated cord blood and haploidentical related transplantation are able to produce results similar to matched sibling BMT; thus, most patients should now have multiple acceptable donor options [25]. In this study, 1 recipient received an autologous transplant and all other potential recipients who desired or remained eligible for transplantation had an alternative donor identified (Table 2).

These data demonstrate that pathologic evaluation of the marrow with morphologic, flow cytometric, molecular, and cytogenetic studies identifies a significant number of marrow abnormalities in selected potential donors. The recent success of alternative donor transplantations would appear to make screening potential donors to rule out transplantable diseases even more important, and adopting this extended screening strategy may help to optimize patient outcomes in BMT.

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Conflict of interest statement: There are no conflicts of interest to report.

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Pretransplant Immunosuppression followed by Reduced-Toxicity Conditioning and Stem Cell Transplantation in High-Risk Thalassemia: A Safe Approach to Disease Control

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Patients with class 3 thalassemia with high-risk features for adverse events after high-dose chemotherapy with hematopoietic stem cell transplantation (HSCT) are difficult to treat, tending to either suffer serious toxicity or fail to establish stable graft function. We performed HSCT in 18 such patients age ≥7 years and hepatomegaly using a novel approach with pretransplant immunosuppression followed by a myeloablative reduced-toxicity conditioning regimen (fludarabine and i.v. busulfan [Flu-IV Bu]) and then HSCT. The median patient age was 14 years (range, 10 to 18 years). Before the Flu-IV Bu + antithymocyte globulin conditioning regimen, all patients received 1 to 2 cycles of pretransplant immunosuppression with fludarabine and dexamethasone. Thirteen patients received a related donor graft, and 5 received an unrelated donor graft. An initial prompt engraftment of donor cells with full donor chimerism was observed in all 18 patients, but 2 patients developed secondary mixed chimerism that necessitated withdrawal of immunosuppression to achieve full donor chimerism. Two patients (11%) had acute grade III-IV graft-versus-host disease, and 5 patients had limited chronic graft-versus-host disease. The only treatment-related mortality was from infection, and with a median follow-up of 42 months (range, 4 to 75), the 5-year overall survival and thalassemia-free survival were 89%. We conclude that this novel sequential immunoablative pretransplantation conditioning program is safe and effective for patients with high-risk class 3 thalassemia exhibiting additional comorbidities.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the sole available curative therapy for patients with severe β-thalassemia, providing potential cure in approximately 80% of recipients [1]. However, several reports have suggested the existence of a subset of patients with worse outcomes. This subgroup includes older patients with normal organ damage due to iron overload and/or evidence of immunization to donor histocompatibility antigens

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